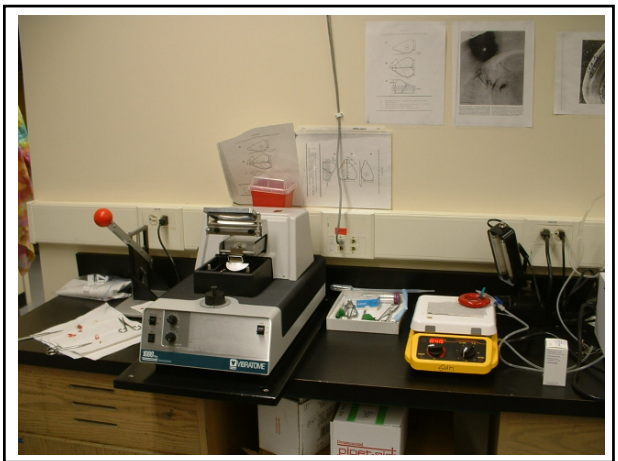
Dissociating brain cells from postnatal GFP expressing mouse

Cell Physiology lab /No. *
Chunzhao Zhang BSc.

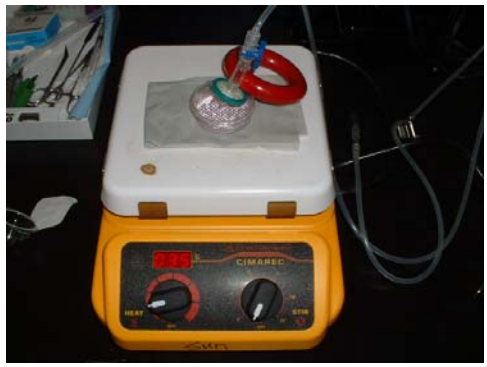


Before Dissection:

- 1. Place 10 mL Papain Buffer (made in advance) in a 60 mm petri dish under CO2/O2 gas (using fresh 0.22um filter) on wet paper towels in 37C heat block to equilibrate about 15 minutes.
- 2. Put a 500ul drop Đ PBS w/o Ca++/ Mg++ in a 60mm petri dish.
- Set up dissection area.

-Dissection:

Cut off head, skin, and skull. Remove brain cortical hemispheres. Transfer the brain to the 500uL Đ PBS w/o Ca++/Mg++ drop and dice the tissue into about 1mm square with a #10 scalpel blade.



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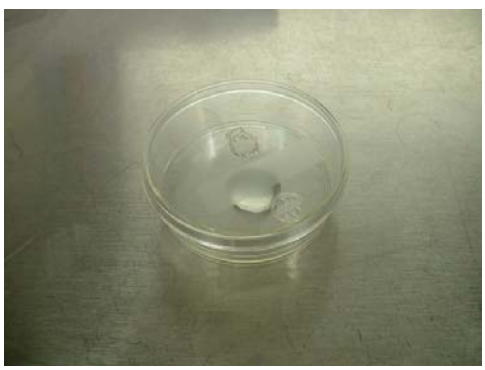
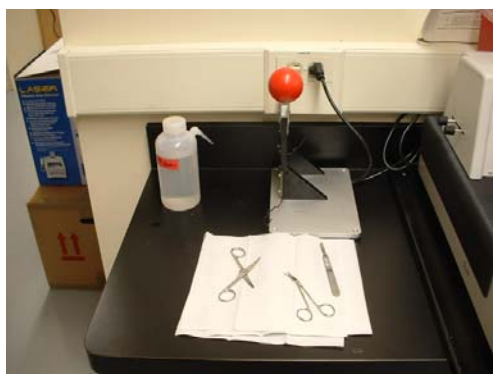


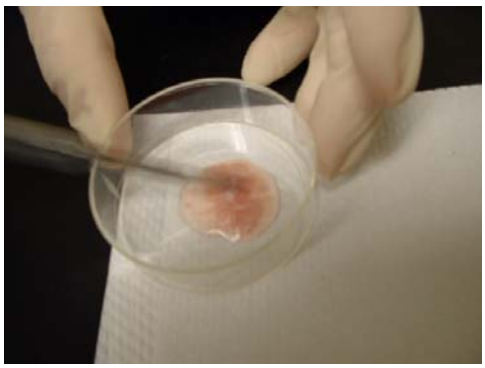
Put a 500ul drop Đ PBS w/o Ca++/ Mg++ in a 60mm petri dish.



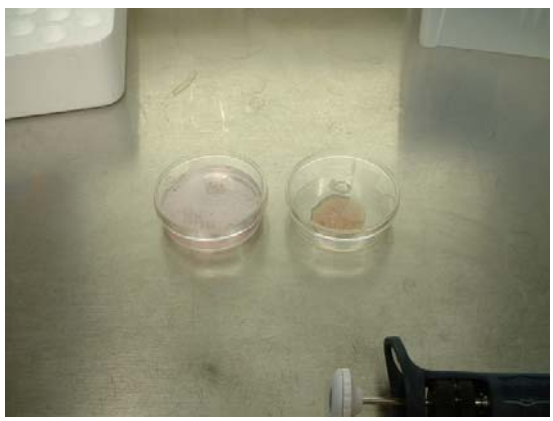
Set up dissection area.

Cut off head, skin, and skull. Remove brain cortical hemispheres. scalpel blade.

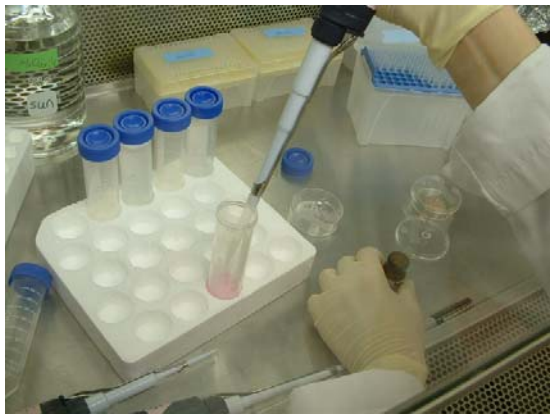
Transfer the brain to the 500uL D PBS w/o Ca++/Mg++ drop and dice the tissue into about 1mm square with a #10



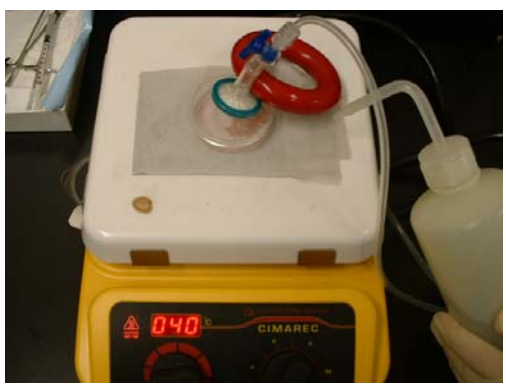
dice the tissue into about 1mm square with a #10 scalpel blade.



Put equilibrated Papain Buffer in a 15 mL tube, add 200 units papain and place at 37C. Weigh out and add 2mg L-cysteine then filter through a 0.22um syringe filter and add 200uL DNase.



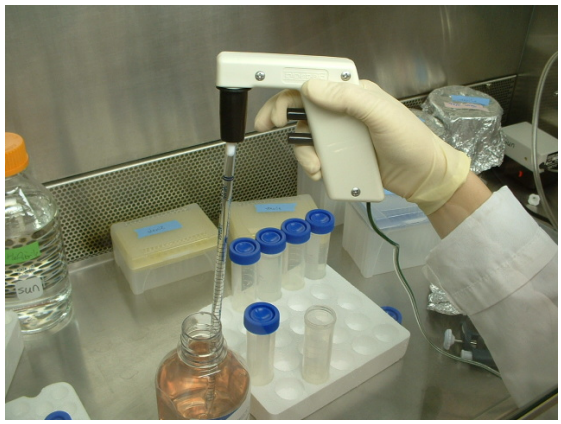
Add this solution to the diced brain dish and place this under the CO/O2 gas on 37C warmed paper towels for 80 minutes, shaking gently every 15 minutes.



Add this solution to the diced brain dish and place this under the CO/O2 gas on 37C warmed paper towels for 80 minutes, shaking gently every 15 minutes.

Meanwhile, make Lo Ovo, Hi Ovo and panning buffer.

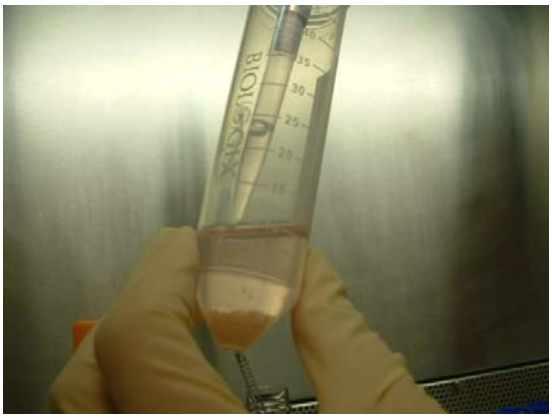
- LoOvo: 9ml DPBS + 1 ml LoOvo + 200ul DNase, 10ml in total.
- HiOvo: 5ml DPBS + 1 ml Hi Ovo, 6 ml in total.
- panning buffer: 13.5ml DPBS + 1.5 ml DPBS/0.2%BSA + 150 ul Insulin.

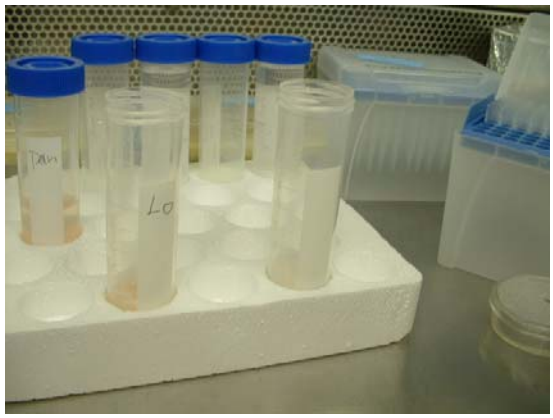


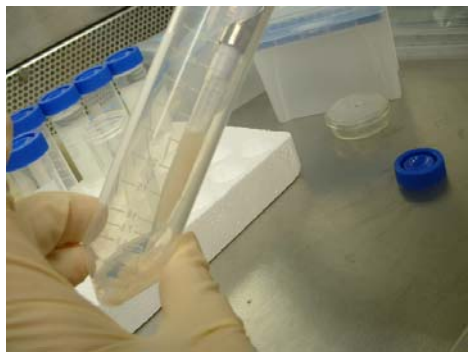
Trituration:

- Transfer dissociation into 1 50ml tube and let settle.
- Remove Papain Buffer and gently add 2 mL Lo Ovo to tube. Let settle and aspirate.
- Add 2 mL Lo Ovo to tube and triturate with 5 mL pipette gently (pipette up and down, let settle, remove 1mL and place in 15 mL tube, add 1mL fresh Lo Ovo and repeat with 5 mL pipette once more.

From the third time, switch to blue tip to do the trituration until no chunks left).

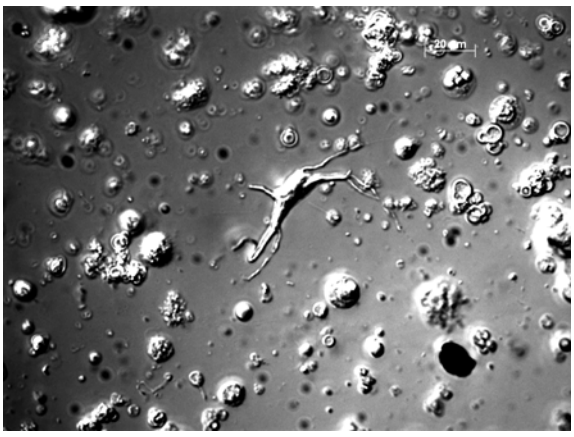


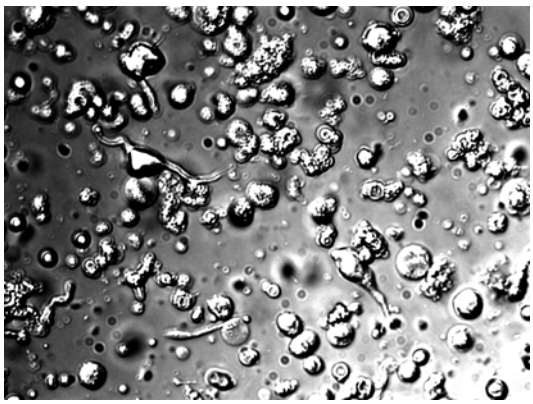




From the third time, switch to blue tip to do the trituration until no chunks left).

When finished, spin 1000RPM, 15 minutes, twice, in Low OVO and high ovo, respectively .





Cell Counting with a Hemocytometer

1) Gently clean hemocytometer and its coverslip with a dry Kimwipe. Place hemocytometer on microscope and place coverslip on hemocytometer.

2) Add 100 ul of an 0.1% trypan blue solution to 100 ul of the cell suspension in a 1.5 ml eppendorf tube. Gently mix with a 200 ul pipetman several times. (Be sure to add the trypan blue to the cells just before the count, because after several minutes it is toxic to the cells.)

3) Add cell suspension onto the hemocytometer using the pipetman. Do not over- or under- fill.

4) Count both the number of cells that exclude trypan blue (viable) and the number of cells that do not exclude trypan blue (they are blue and not viable). Count both grids. At low power (10X objective), the grid looks like a 3 x 3 grid. At high power (20x objective), the center box can be seen to be further subdivided into a 5 x 5 grid (and each of these is further subdivided). If there are many cells, just count the center box of the 3 x 3 grid, otherwise count the entire 3 x 3 grid. When cells touch the outside lines of the grid, only count the left and top lines.

5) Add your counts from each grid together to compensate for the 1:1 dilution from adding trypan blue to the cells.

6) To determine the number of cells per ml:

The volume of each box in the 3 x 3 grid is 0.1 cubic mm. Therefore if the entire grid was counted, multiply the count by 10/9 x 103. If only the center grid was counted, multiply the count x 10 x 103 to give the total number of cells/ml. This gives the cells/ml in the original cell suspension. Multiply this number by the number of total mls in the cell suspension to give the absolute number of cells obtained from the dissociation.

7) Clean the hemocytometer immediately, by gently wiping off with a Kimwipe.

Summary

At low cell density (all 9 boxes of grid counted),

Total cell yield = (total cells both grids) x 10/9 x 103 x # mls.

At high cell density (only 1 of 9 boxes counted),

Total cell yield = (total cells both grids) x 10 x 103 x # mls.